We have assessed the tumouricidal activity of UVI5008, *in vitro* in a panel of cancer cell lines as well as *ex vivo* in leukemia patient's blasts. Our results indicate that UVI5008 inhibits proliferation by inducing apoptosis of these cells. *In vitro* enzymatic assays showed that UVI5008 can inhibit the activity of class I & II HDACs, Sirtuins and DNA methyltransferase.
We could also show that UVI5008 exerts its antitumour effect *in vivo* in xenografted tumours and in mammary tumour model. This activity is p53 independent and selective for cancer cells, without significant toxicity to normal cells. Growth inhibition is achieved by increased acetylation and induction of TNF related apoptosis inducing ligand (TRAIL) and Reactive oxygen species (ROS). We have also observed reduced methylation and re-expression of p16/INK4 and Retinoic acid receptor-beta 2, two tumour suppressor genes usually silenced in tumour cells by promoter hypermethylation.
Taken together, our data strongly suggest that targeting of multiple signaling pathways by a single drug is a feasible and attractive paradigm for new cancer therapies.

267 The effect of bevacizumab on intratumoural angiogenesis of malignant fibrous histiocytoma in animal model

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Background: Vascular endothelial growth factor (VEGF) is considered to be a key mediator among the angiogenic growth factors causing tumour growth and metastasis, hence the development of anticancer drugs targeting angiogenesis and clinical trials have been widely conducted. Bevacizumab is one of the specific inhibitors for angiogenesis and a neutralizing antibody against vascular endothelial growth factor (VEGF), has recently been used as a drug against malignant tumours. In this study, we evaluated an effect of bevacizumab against malignant fibrous histiocytoma (MFH) in the animal model.
Material and Methods: MFH cell line, Nara H, was used. We injected Nara H cells (1.2×10^7) subcutaneously to the dorsal area of nude mice. After implantation, we measured body weight and tumour dimensions twice a week. Tumour volume was calculated according to the formula $V = \pi/6 \times a^2 \times b$, where *a* and *b* represent the shorter and the longer dimension of the tumour.
Effect of bevacizumab on tumour growth: Mice were randomly divided into treatment group (n = 25) and control group (n = 25). We started treatment with bevacizumab (2 mg/kg) or PBS for each group, twice a week, intraperitoneally. We measured body weight and calculated tumour volume and survival rate for 8 weeks.
Immunohistochemical analysis: Nineteen mice received intraperitoneal injection with bevacizumab or PBS twice a week (treatment group (n = 10) and control group (n = 9)). After 18 days, all tumours were removed and immunohistochemical analysis was performed with Factor-VIII and VEGF antibodies to evaluate microvessel density (MVD) and VEGF expression.
Results: *Tumour growth was significantly inhibited by bevacizumab:* We did not find a difference in body weight between two groups. Tumour volume was significantly decreased in treatment group compared with control group after 16 days treatment. At the end of experimental period, the mean tumour volume of treatment group and control group were 2.7×10^{-5} m^3 and 1.4×10^{-5} m^3, respectively. There was no significant difference in survival rate between two groups, however survival rate of treatment group was higher than that of control group (76.6% with treatment group and 59.7% with control group).
Bevacizumab significantly decreased MVD but not VEGF expression: There was no significant difference in VEGF expression between two groups. MVD was significantly decreased in treatment group. The mean MVD value was 4.2 in treatment group and 7.2 in control group (p = 0.005). We also found a significant correlation between tumour volume and MVD in treatment group (p = 0.02, r = 0.53).
Conclusions: In this study, bevacizumab significantly inhibited tumour volume and intratumoural MVD of MFH in vivo, and there was a significant correlation between tumour volume and MVD in treatment group. These results suggest that bevacizumab may suppress tumour growth of MFH via inhibiting intratumoural micro vessel formation and that bevacizumab may be a novel therapeutic agent for MFH.

268 Expression profile of genes influencing the efficiency of taxanes in breast cancer therapy

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Background: Taxanes have been successfully used in the therapy of various cancers, mainly breast and ovarian cancers. However, multidrug resistance (MDR) of tumour cells to anticancer drugs (i.e. taxanes) represents a problem in the cancer chemotherapy. MDR is a significant cause of the failure of chemotherapy in tumours with inherent or acquired resistance due to enhanced expression of ABC transporters, especially P-glycoprotein (encoded by *ABCB1*). Together with the transporter-mediated resistance, alterations in apoptosis induction by taxanes may be related to tumour resistance, but molecular mechanism(s) is not fully understood. One of causes of the resistance to apoptosis can be the role of caspase-2, mainly different expression of caspase-2S (antiapoptotic) and caspase-2L (proapoptotic) isoforms.
Material and Methods: Expression profile of ABC transporter genes (*ABCB1, ABCC1* and *ABCC2*) was evaluated in 33 breast cancer patients treated by neoadjuvant chemotherapy. Gene expression was quantified in paired tumour and non-tumour breast tissue samples using real-time PCR method with absolute quantification and normalization to cyclophillin A as a housekeeping gene. In addition, the particular isoforms of caspase-2 gene were detected using RT-PCR method.
Results: Caspase-2S/L isoforms as well as ABC transporter genes were identified in examined subjects. ABC transporters were expressed in majority of cases with inter-individual variability in their expression. The levels of expression were as follows; *ABCC1* > *ABCB1* > *ABCC2. ABCC1* was up-regulated in 60% of all tumours, while opposite was observed for *ABCC2. ABCB1* was up-regulated in about half of tumour samples (51.5%).
Conclusions: High expression of *ABCB1* gene in particular tumour samples seems to be important prognostic factor, because patients with high *ABCB1* expression treated with P-gp substrates anthracycline- or taxane-containing regimens had significantly shorter disease-free survival than those treated by other regimens (P = 0.031). In addition, our findings indicate that gene *ABCC1* is, due to its high expression in breast tumour tissue, another potential candidate gene for breast cancer resistance. The presence of antiapoptotic isoform caspase-2 (caspase-2S) seems relevant for additional decrease of efficiency of taxane-based regimen due to the inhibition of the apoptosis in cancer cells caused by taxanes.
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269 Intermittent treatment schedules with rapamycin against malignant glioma xenograft model

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The main aim of this study was to establish a drug dose and treatment schedule to enhance the efficacy in the treatment of malignant glioma xenograft model with rapamycin, improving the survival time of animals bearing brain tumours.
In vitro toxicity experiments were carried out with established human glioblastoma multiforme cell lines U87-MG and U251-MG. The drug activity at different concentrations and times of incubation was studied. Survival studies were performed using the well-established intracranial glioblastoma tumour xenograft model U87MG. Animals were treated intraperitoneally using different schedules and doses in order to compare the efficacy and the benefits of each system. In vitro and in vivo studies of the mammalian target of rapamycin, mTOR, were also accomplished in order to know the activity of rapamycin in each case and apply this knowledge to improve the treatment.
The efficacy of rapamycin in the in vitro and in vivo experiments was found to be no dose-dependant. Animals in the control group had a median survival (MS) of 14 days. Animals treated with rapamycin at 10 mg/kg once a week had a MS of 45.2 days. An important improvement in the survival was not observed when the dose increased to 25 mg/kg, MS = 48.2 days, (P = 0.0271 pair wise comparison). Comparing restricted treatment of rapamycin (days 7, 8, 9, 15, 16 and 17 after tumour implantation) with intermittent treatment (every 5 days) using the same dose (10 mg/kg) we observed a significant improvement in survival. Restricted treatment had a MS = 32.5 days and intermittent treatment showed a MS = 55 days, (P = 0.00313 pair wise comparison).
Our results suggest that a treatment with intermittent intraperitoneal injection is more effective than a daily injection for a restricted period. Intermittent injections allow to keep mTOR pathway inhibited for a longer time. When rapamycin starts to be cleared due to the activity of cytochrome P450 and the stability of the FKBP-Rapamycin complex starts to become weak, a new administration of rapamycin prolongs mTOR pathway inhibition. We demonstrated also, with this study that is more important to keep the activity of rapamycin in the brain than to provide a higher concentration. The response to every 5 days treatment (10 mg/kg) was better than every 7 days treatment (25 mg/kg) even at lower dose, because the administration was provided when mTOR pathway seems to recover the activity. The efficacy of intermittent treatment schedules suggests a therapeutic window reducing the toxicity due to the drug, decreasing the dose to the minimum effective dose that is able to inhibit the mTOR pathway. We conclude that intermittent doses of rapamycin may be an effective treatment option for malignant gliomas.